# A COMPARISON OF THE OXYGEN CONSUMPTION OF UNFERTILIZED AND FERTILIZED EGGS OF *FUNDULUS HETEROCLITUS*.

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Since Warburg (1) in 1908 measured the oxygen consumption of *Arbacia* eggs and observed the marked increase following fertilization, the oxidation processes in marine eggs and embryos have been carefully investigated. The rate at which the oxygen is removed from the surrounding air or sea water has been correlated with the stages in development. Thus Shearer (2) measured the oxygen consumption during fertilization of Echinoderm eggs, and found a decided increase upon the addition of the sperm; "more oxygen is taken up in the first minute of the process than at any subsequent interval of the same time." In another article Shearer (3) states that, in the first hour of development, the fertilized egg consumes six to seven times as much oxygen as the unfertilized egg. In the star fish egg, however, according to Loeb and Wastenys (4) there is no increase in the oxidation rate after fertilization.

The rate of oxygen consumption is also correlated closely with heat production. Rogers and Cole (5) in their work on *Arbacia* eggs have shown how the heat production varies before, during, and after fertilization; according to them "the rate of heat production at the instant of fertilization is ten to twelve times that of the unfertilized egg."

The literature upon this subject reports work done almost exclusively upon invertebrate eggs. Apparently no previous study of the influence of fertilization upon respiratory rate has been made on any vertebrate egg. Scott and Kellicott (6) and Hyman (7), who have measured the oxygen consumption of *Fundulus* embryos at various stages of development, made no observations on the respiration during the first two hours after fertilization, and secured no information as to the influence of fertilization

itself. The present study represents an attempt to secure such information. It has been possible to show by several methods that fertilization markedly increases the oxygen consumption of the eggs of *Fundulus heteroclitus*. The time relations of this increase are of some interest.

### 1. Winkler Method for Determination of Dissolved Oxygen.

The Winkler method as applied to this problem was employed in the manner described by Amberson, Mayerson and Scott (8). 600 eggs were placed in 500 cc. of sea water in each of two small Erlenmeyer flasks. Samples for analysis were withdrawn through siphons. The water surface was covered with paraffin oil to minimize the diffusion of new oxygen from the air into the water. The sea water was analyzed for dissolved oxygen previous to experimentation; the initial sample was withdrawn after twenty to forty minutes. In order to secure successive determinations of the dissolved oxygen during an extended time, it was necessary to adopt a micro-Winkler method as suggested by Lund (9). For these analyses small vials of 6.5 cc. capacity were used. Fig. 1 represents the graph resulting from plotting the data shown in Table I. below. The values for the amount of oxygen consumed during a given period are obtained by subtracting the amount remaining in the sea water at the end of that period from the amount originally present in the sea water used for the experiment.

Table I.

| Time. | Sea Water Originally Contains 5.1 cc. Oxygen per Liter. | | | |
|---|---|---|---|---|
| | Unfertilized Eggs. | | Fertilized Eggs. | |
| | $O_2$ Remaining. | $O_2$ Consumed. | $O_2$ Remaining. | $O_2$ Consumed. |
| 20 min. | 5.0 | 0.1 | 4.7 | 0.4 |
| 45 min. | 4.9 | 0.2 | 4.5 | 0.6 |
| 7 hrs. | — | — | 3.7 | 1.4 |
| 10 hrs. and 35 min. | 4.6 | 0.5 | 3.4 | 1.7 |
| 25 hrs. and 25 min. | 3.8 | 1.3 | 2.8 | 2.3 |
| 31 hrs. and 25 min. | 3.1 | 2.0 | 1.8 | 3.3 |

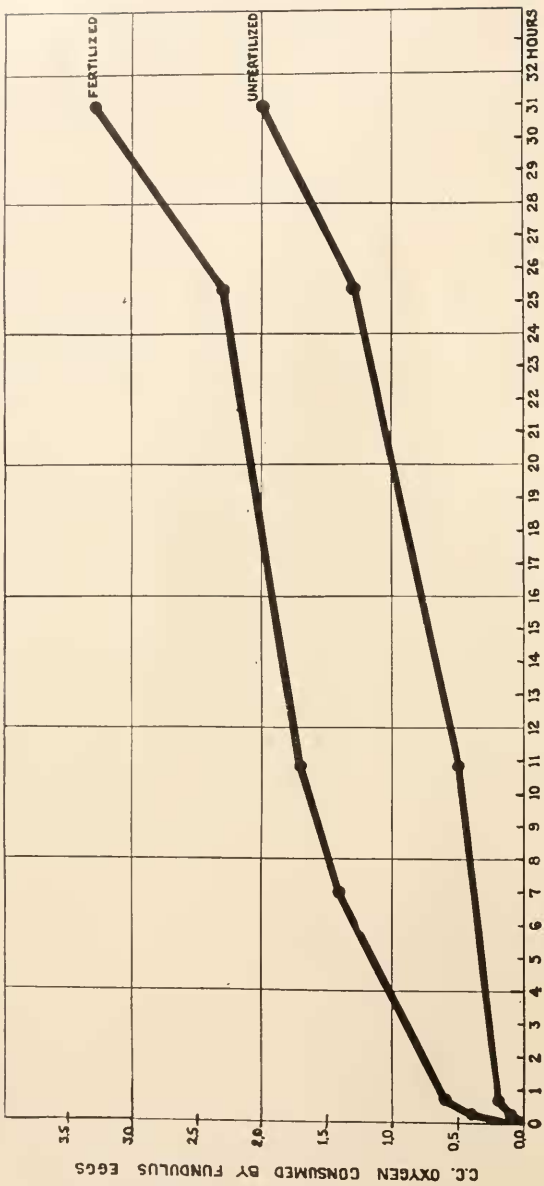


FIG. 1. Results obtained from Winkler Method. The amount of dissolved oxygen found at each successive time is subtracted from 5.1 c.c., the amount of dissolved oxygen originally in the sea water used for the experiment. The total amount of oxygen consumed is thus determined and is plotted against time.

It is to be noticed that the fertilized eggs had at the time of each determination consumed more oxygen than the unfertilized eggs. Data from similar experiments, as shown in Table II., show that the rate of oxygen consumption is most rapid during the first two hours following fertilization.



TABLE II.

| Time. | Sea Water Originally Contains 5.2 cc. Oxygen per Liter. | | | |
|---|---|---|---|---|
| | Unfertilized Eggs. | | Fertilized Eggs. | |
| | $O_2$ Remaining. | $O_2$ Consumed. | $O_2$ Remaining. | $O_2$ Consumed. |
| 20 min. . . . | 5.1 | 0.1 | 4.7 | 0.4 |
| 1 hr. . . . . | 5.0 | 0.2 | 4.4 | 0.8 |
| 2 hrs. . . . . | 4.9 | 0.3 | 4.0 | 1.2 |
| 4 hrs. . . . . | 4.8 | 0.4 | 3.9 | 1.3 |

## 2. MICRO-RESPIROMETER METHOD FOR DETERMINATION OF OXYGEN.

The type of micro-respirometer that was used for the study of oxygen consumption by the *Fundulus* eggs is one that has been devised by W. O. Fenn for similar studies of *Arbacia* eggs. A small glass bottle with a ground glass neck is fitted with a ground glass stopper which is connected with a horizontal fine-bore manometer provided with a centimeter scale. In the center of the bottom of the bottle is a small compartment into which 0.5 cc. of 15 per cent. NaOH is introduced; the eggs to be studied are placed in the space surrounding the compartment. The NaOH serves to absorb the $CO_2$ produced by the eggs. A small drop of kerosene is introduced into the manometer and its movement across the tube from the outer to the inner end indicates both the amount of oxygen consumed and the rate of the process.

Five cc. of sea water, containing fifty *Fundulus* eggs, were pipetted into the micro-respirometer. Two micro-respirometers were used so that experiments on unfertilized and fertilized eggs could be carried out at the same time under identical conditions. The constants of each apparatus were found by calibration of the respective manometers. The micro-respirometers were immersed in a bath of running sea water; the temperature for all of the

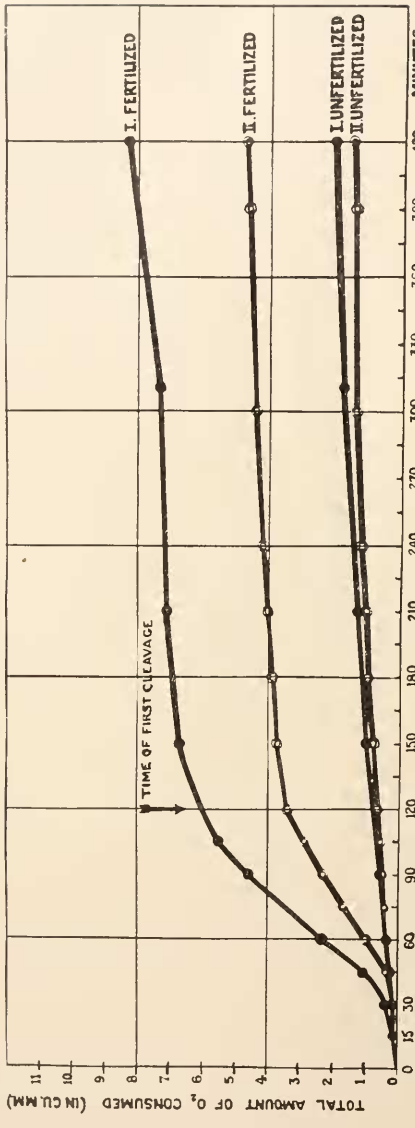


FIG. 2. Results obtained by the micro-respirometer method, showing total amounts of oxygen consumed by fertilized and unfertilized eggs at successive periods. Cubic mm. of oxygen are plotted against time. Experiments I. and II. are chosen to show typical results.

experiments proved to be 20.2 ± .6° C. The readings of the meniscus of the kerosene drop were taken at intervals of fifteen minutes. Over twenty series were run, but in only the last five experiments were the temperature variations observed with a Beckman differential thermometer.

Figure 2 shows typical curves for the results obtained. In Experiment I. the number of cubic millimeters of oxygen consumed by the fertilized eggs is a little less than twice the corresponding amount in the case of the fertilized eggs in Experiment II. Nevertheless both curves show the same marked increase in oxygen consumption 45 minutes after fertilization. This increased oxygen utilization reaches a maximum during the period from 60 to 90 minutes following fertilization. From this time on, the amount of oxygen consumed per unit time falls so that the rate of utilization approximates that for the unfertilized eggs. It would appear, therefore, that the oxygen requirements of the unfertilized *Fundulus* eggs are increased by fertilization. The time relations of this increase are of theoretical interest; they are indicative of some oxidation process occurring within the egg for which an increased oxygen intake is a necessity. To follow the development in relation to the time, two control sets of 50 *Fundulus* eggs were placed in sea water and the stages of development were traced by microscopic inspection. It was found that the increased rate of oxidation occurs at a time before and during the appearance of the groove in the surface of the blastodisc which initiates the first cleavage. The subsequent cleavages evidently do not require such a marked rate of oxygen intake. A single run with twenty 9-day old *Fundulus* embryos revealed a later rise in the oxygen consumption which can be correlated with the marked rise in the rate which Scott and Kellicott found to occur at the time circulation is established.

To show still further the peculiarity of the time relations, the average amount of oxygen consumed per 10 minutes was calculated from the data of Experiment I. and is shown in Fig. 3. The difference in the rates of the fertilized and unfertilized eggs is markedly contrasted.

A few experiments were carried out by a third method and gave results that checked qualitatively with the two mentioned

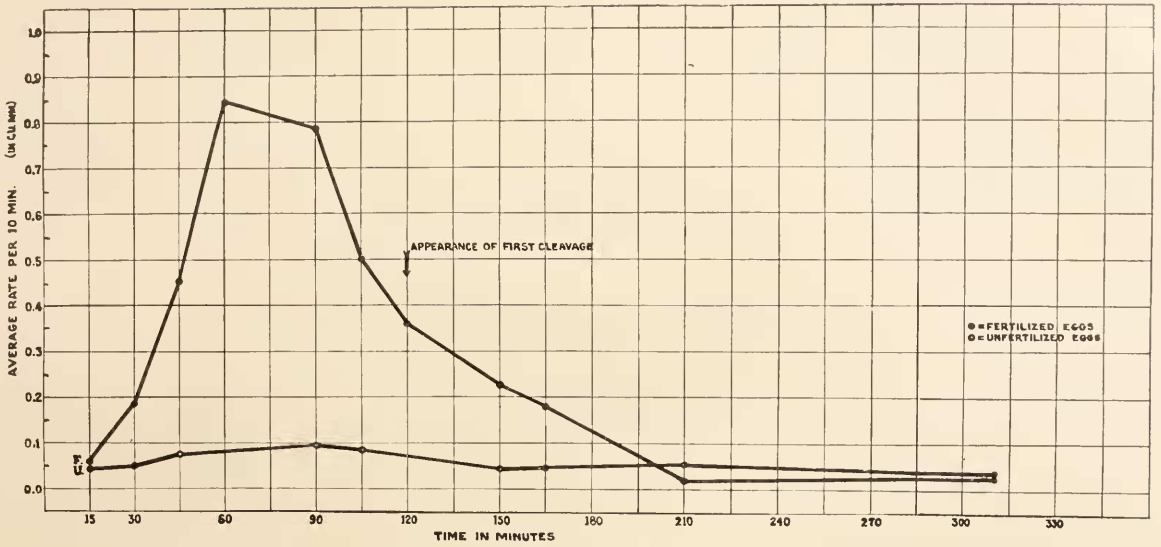


FIG. 3. Respiratory-rate-time graph. The marked increase in the rate of oxygen consumption after fertilization is shown to precede the time of first cleavage. The rate of oxygen consumption of unfertilized eggs is practically constant.

above. This method involved the analysis of air above 25 cc. of water in a 100 cc. Haldane gas collector. At the beginning of the experiment 200 *Fundulus* eggs were pipetted into each of two collectors with the sea water, and the water was equilibrated with atmospheric air which filled the vessel. The collectors were then sealed, immersed, and rotated in a bath of running sea water for two hours. More oxygen was found to have been lost from the sample of air taken from the collector containing the fertilized eggs than from that containing the unfertilized. This method proved to be only approximate as the rotation caused the eggs to stick together in a clump and normal development did not take place. The data secured gave a qualitative confirmation of the more accurate results obtained by the two other methods.

## Summary.

By three methods it has been shown that the oxygen consumption of the eggs of *Fundulus heteroclitus* is greatly increased after fertilization. This increased rate of oxygen consumption is at its maximum from 60 to 90 minutes after the addition of the sperm, in a period immediately preceding the first cleavage. The oxygen consumption then falls to a level practically identical with that of the unfertilized eggs.

The writer wishes to express her appreciation to Dr. W. R. Amberson and Dr. W. O. Fenn for their suggestions, and to Mr. J. O. Pinkston for his assistance in the oxygen determinations.

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